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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
Esther H. CHANG et al.	)	
Serial No. 09/856,270	)	Examiner: Liping Chen
Filed: May 18, 2001	)	Group Art Unit: 1632
For: SYSTEMIC VIRAL/LIGAND GENE DELIVERY SYSTEM AND GENE THERAPY	)	

DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Esther Chang, declare that:

1. I am the same Esther Chang named as an inventor on the above-referenced patent application.

2. I received a B.A. degree in biology from Fu Jen University in Taiwan in 1968 and a Ph.D. in microbiology from Southern Illinois University in 1974. From 1982-1994 I held the positions of Assistant Professor, Associate Professor, and then Professor in the Department of Pathology, Uniformed Services University of the Health Sciences in Bethesda, MD. I also was a Research Professor in their Department of Surgery and the Director of their Tumor Biology Program. From 1994-1996 I held the position of Professor of Surgery (Research), Division of

Otolaryngology/Head and Neck Surgery in the Department of Surgery at Stanford University Medical Center. Since 1996, I have held the position of Professor of Surgery (Consultant) there. I currently also hold the positions of Professor of Otolaryngology, Department of Otolaryngology/Head & Neck Surgery and Professor of Oncology and Otolaryngology, Departments of Oncology and Otolaryngology, at the Georgetown University Medical Center, Lombardi Cancer Center, and have held those positions since 1996 and 1999, respectively. A copy of my curriculum vitae is attached hereto.

3. I have read the Office Action issued by the U.S. Patent and Trademark Office on August 14, 2002, and understand the grounds of rejection set forth therein. In one rejection the examiner asserted that the scope of the claims had not been enabled by the specification. Specifically, he asserted that although the claims provide that the cell-targeting ligand can be a protein, peptide, hormone, antibody or antibody fragment, the examples in the application only illustrate the use of transferrin as the ligand.

4. Studies have been carried out by me or under my direction which demonstrate that a variety of molecules can be used in the method of this invention and will non-covalently bind directly to virus particles. More specifically, we have illustrated the usefulness of the present invention using both a

single chain antibody fragment and a protein, epidermal growth factor protein (EGF), as the cell-targeting ligand.

In the experiments, a single chain Fv fragment of the anti-transferrin receptor monoclonal antibody (TfRscFv) or the EGF protein was mixed with either replication deficient adenovirus serotype 5, Ad5LacZ, containing the *E. coli* LacZ gene under control of the CMV promoter ($2.2x10^{12}$ particles/ml in PBS plus 3% sucrose) or replication deficient adenovirus serotype 5, Ad5p53, carrying the normal human p53 gene under control of the CMV promoter ($2.4x10^{12}$ particles/ml in PBS plus 3% sucrose). Using the method described in Example 1 of the application, the TfRscFv or the EGF first was diluted to 0.1 mg/ml in 10 mM HEPES buffer, pH 7.4, then added to 50 µl HEPES buffer. Virus then was added to the tubes so that the ligand to virus ratio was 5000 ligand molecules/virion. The mixtures were incubated at room temperature for 10-15 minutes and then 150 µl EMEM without serum were added to each tube.

In vitro adenoviral transduction was carried out using TfRscFv or EGF as the targeting ligand. 5×10^4 JSQ-3 cells/well were plated in a 24 well plate. 24 hours later, the cells were washed once with EMEM without serum, 0.3 ml EMEM without serum or antibiotics were added to each well. The Ad5 LacZ, TfRscFv-Ad5LacZ, EGF-Ad5LacZ complexes, as well as Tf-Ad5LacZ as a positive control, were added to duplicate wells. After two hours

incubation at 37°C, 5% CO_2, with occasional rocking, 0.5 EMEM with 20% serum were added to the wells. After 24 hours in culture, the cells were washed once in PBS and lysed in 1X reported lysis buffer (Promega). The cell lysates were treated with 100 µl of 130µM o-nitrophenyl-β-galactopyranoside in 20 mM Tris (pH 7.5) containing 1 mM $MgCL_2$ and 450 nM β-mercaptoethanol at 37°C for 30 minutes. The reaction was stopped by the addition of 150 µl/well of 1 M Na_2CO_3. The absorbency was determined at 405 nm. Purified β-galactosidase (Boehringer) was used to make a standard curve. The results were expressed as miliUnit (mU) of β-galactosidase equivalent per mg of total protein.

JSQ-3 cells, seeded as above also were virally infected with the Ad5p53, EGF-Ad5p53 complexes, as described above. simultaneously with the viral infection, the cells were transfected (Xu et al. *Human Gene Therapy* 10:2941-2953 (1999)) with a Tf-cationic liposome DNA complex in which the DNA was a plasmid carrying the luciferase gene under control of the p53 responsive MDM2 promoter (BP100). thus, the efficiency of the viral infection can be expressed as the level of luciferase activity in the cells. Twenty four hours after infection/transfection, the wells were washed once with PBS, and 200µl of 1X reporter lysis buffer (Promega) was added to each well. After shaking at 200RPM for 10-15 minutes at room temperature on an orbital shaker, the plates were frozen and

thawed three times at -80°C to complete the lysis. The cell lysate was transferred to tubes, vortexed for 10-15 seconds and centrifuged at 12,000x g for two minutes at 4°C. Ten µl of the lysate were mixed with 50µl of Luciferase Assay reagent (Promega) and immediately read on a luminometer. Protein was determined using the micro BCA protein detection kit(Pierce). The results were expressed as relative light units/µg of protein.

5. Table 1, attached, shows the results of the Ad5LacZ transfection. the non-covalent direct binding of either the TfRscFv or EGF to the adenoviral vector resulted in a significant increase in transfection efficiency over that of the untargeted adenovirus. Moreover, the level of increase with these other types of ligands was similar or even better, in the case of EGF, than that obtained when Tf was used in the admixture.

The results of the luciferase assay for p53 expression after targeted delivery by the admixtures of EGF-Ad5p53 mirror those described above for β-galactosidase (Table 2). Using the method of the present invention, the EGF protein clearly was able to non-covalently, directly bind to the Adp53 resulting in tumor cell targeting and increased viral p53 transduction efficiency as evidenced by the significant increased level of luciferase activity as compared to the unliganded virus.

6. These results demonstrate the molecules other than transferrin, such as antibodies and proteins, can noncovalently and directly complex adenovirus and significantly enhance gene transduction. In view of these results, it is my expectation that viruses other than adenoviruses also can be complexed with such ligands to enhance gene transduction.

7. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Codes, and that such wilful false statements may jeopardize the validity of the application and any patent issuing thereon.

Esther Chang, Ph.D.

12/09/02
Date

CURRICULUM VITAE

10/7/2002

PERSONAL

Name: Esther H. Chang
Place of Birth: Chungking, China
Citizenship: U.S. Citizen
Marital Status: Married with 1 daughter (Harford)
Work Address: Departments of Oncology & Otolaryngology
Georgetown University Medical Center
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EDUCATION

Fu Jen University, Taiwan	B.A.	1968	Biology
Southern Illinois University	Ph.D.	1974	Microbiology

PROFESSIONAL APPOINTMENTS

Trainee U.S. Naval Medical Research Unit No. 2 Taiwan	1967 - 1968
Research Assistant Southern Illinois University	1968 - 1971
Teaching Assistant in Immunology and Virology Southern Illinois University	1971 - 1972
Research Associate Southern Illinois University	1972 - 1973
Special Dissertation Fellow Southern Illinois University	1973 - 1974
Visiting Fellow National Institutes of Health	1974 - 1977
Visiting Associate National Institutes of Health	1977 - 1978
Cancer Expert National Cancer Institute	1978 - 1982
Assistant Professor Department of Pathology Uniformed Services University of the Health Sciences	1982 - 1983
Associate Professor and Coordinator for Medical Genetics Curriculum	1983 - 1988

Department of Pathology Uniformed Services University of the Health Sciences Professor, Department of Pathology Research Professor, Department of Surgery Coordinator for Medical Genetics Curriculum Director, Tumor Biology Program Uniformed Services University of the Health Sciences	1988 - 1994
Professor of Surgery (Research) Division of Otolaryngology/Head & Neck Surgery Department of Surgery Stanford University Medical Center	1994 - 1996
Professor of Surgery (Consultant) Division of Otolaryngology/Head & Neck Surgery Department of Surgery Stanford University Medical Center	1996-Present
Professor of Otolaryngology Department of Otolaryngology/Head & Neck Surgery Georgetown University Medical Center Lombardi Cancer Center	1996-Present
Professor of Oncology and Otolaryngology Departments of Oncology and Otolaryngology Georgetown University Medical Center Lombardi Cancer Center	1999-Present

HONORS AND OTHER SPECIAL RECOGNITION

Honor Society of Phi Kappa Phi	1972
Special Dissertation Fellow Southern Illinois University	1973 - 1974
Author, two papers in 100 most-cited papers in Life Sciences, Current Contents, November 5, 1984	1982 - 1983
Conference Organizer-International Conference on Molecular Biology of Neoplasia Taipai, Taiwan	1984
Ad Hoc Reviewer for NIH Study Section	1985
One of six awardees, Visiting Scholar Exchange Program, National Academy of Sciences, American Council of Learned Societies and Social Science Research Council	1986 - 1987
Member, Merit Review Committee, USUHS	1987 - 1989
Ad hoc Member, Review Panel for Assessment of Department of Energy research projects on chemical toxicology	1989
Member, Faculty Senate Education Committee, USUHS	1990 - 1991
Member, Editorial Board of Antisense Research and Development	1990 - Present

Member, Steering Committee on Prescribing of Drugs by Military Psychologists	1991
Chairman, Subcommittee for Faculty Resources for the Educational Program, Institutional Self-Study at USUHS	1991 - 1993
Member, Scientific Advisory Committee on Design Study for Life Span Experiments in Mice on Carcinogenesis and Biological Effects of Heavy Charged Particles, NASA	1992 - 1994
Chairman, Subcommittee to Examine Faculty, Middle States Association Reaccreditation Self-Study, USUHS	1992 - 1993
Ad hoc Member, Special Review Committee, Epidemiology, NCI	1992
Author, one Nature paper in top ten most cited papers in medicine Science Watch, September, 1992	1992
Member, Board of Scientific Counselors, Division of Cancer Biology, Diagnosis and Centers, National Cancer Institute	1993 - 1995
Member, NASA Life and Microgravity Sciences and Applications Advisory Committee	1994 - Present
Member, Interim ad hoc Board of Scientific Counselors, National Cancer Institute, NIH	1995 - 1996
Chair, Molecular Genetics Study Section, U.S. Army Breast Cancer Research Program	1997
Chair, Experimental Gene Therapy, Program Committee AACR Annual Meeting	1999
Member, Board of Scientific Advisors, National Cancer Institute	1999 - 2004
Member , Editorial Board of Cancer Gene Therapy	1999 - Present
Member, Scientific Program Committee. Chair, Gene Therapy Program NCI-EORTC-AACR Symposium	1999
Distinguished Alumni, Fu Jen University	1999
10th Lecturer, Stewart Lectureship	2000
Member, NASA Focus Group - National Academy of Sciences, Committee on Science, Engineering, and Public Policy	2000
Member, Committee of Scientific Advisors, United States Military Cancer Institute	2001 – Present
Ad hoc member, Experimental Therapeutics I + II, Study Section, NIH	2002
Organizer, Conference on "Tumor Specific Delivery by Non-Viral Systems" Maui, Feb. 2003 Sponsored by NCI	2002-2003
Approximately 10 annual invited lectures at national and international conferences and academic and research institutes	1982 - Present

DISSERTATION TITLE

Comparative Studies of Growth Patterns of Ganjam Virus in CE, BHK and VERO and *Aedes albopictus* Cells

RESEARCH ACTIVITIES

Undergraduate

Insect tissue culture. Studied growth pattern of insect line cells (Bombyx, Aedes and Antheraea) and adapted two lines into hemolymph-free media. Gained some experience in the growth of Japanese Encephalitis Virus in insect cells and newborn mice.

Graduate School

Arboviruses (Togaviruses). Electron microscopy. Compared the growth of VSV in insect cells and chicken embryo fibroblasts. Determined the viral RNA profiles in each cell line.

Characterized Ganjam Virus, an ungrouped arbovirus.

Postgraduate

RNA tumor viruses - interferon effect. Studied interferon's inhibitory effect on the replication of murine leukemia virus. (In Robert M. Friedman's laboratory, National Institutue of Arthritis, Metabolic and Digestive Diseases, NIH).

Molecular genetics. Cloned and characterized murine leukemia and sarcoma viruses. Investigated the origin and the functional organization of Harvey murine sarcoma virus. Molecularly cloned four DNA fragments containing human homologous sequences of v-*ras* (2 Harvey and 2 Kirsten) and demonstrated their oncogenic potentials. Studied potential human oncogenes. (In Douglas R. Lowy's Laboratory, Dermatology Branch, National Cancer Institute, NIH).

Current

1) Molecular genetic basis of familial cancer syndrome and the involvement of human oncogenes and tumor suppressor genes in carcinogenesis.
2) Modulation of oncogene expression by sequence-specific antisense oligonucleotides.
3) Molecular basis of cellular radioresistance and radioprotection.
4) Tumor Suppressor Gene Therapy for Cancer (Head and Neck, Breast and Prostate)
5) Ligand directed, tumor-targeted liposome-based systemic gene delivery

MEMBERSHIP IN ORGANIZATIONS AND PROFESSIONAL AFFILIATIONS

Honor Society of Phi Kappa Phi	1973-
American Association for the Advancement of Science	1983-
Society of Chinese Bioscientists in America	1988-
The Wound Healing Society	1991-
American Association for Cancer Research	1993-
American Society of Gene Therapy	1997-

PUBLICATIONS - ESTHER H. CHANG

1. R. M. Friedman, **E. H. CHANG**, J.M. Ramseur and M.W. Myers. Interferon-directed inhibition of chronic murine leukemia virus production in cell cultures: Lack of effect of intracellular viral markers. **J. Virol. 16**: 569-574 (1975).

2. R. M. Friedman, J.C. Costa, J.M. Ramseur, M.W. Myers, F.T. Jay and **E. H. CHANG**. Persistence of the viral genome in interferon-treated cells infected with oncogenic or nononcogenic viruses. **The J. Infectious Diseases 133**: A43-A50 (1976).

3. R. M. Friedman, F. T. Jay, **E. H. CHANG**, M. W. Myers, J. M. Ramseur, S. J. Mims, T. J. Triche, and P.K.Y. Wong. Interferon-directed inhibition of chronic murine leukemia virus production in cell cultures. In: Control of Neoplasia by Modulation of the Immune System. (M.A. Chirigos, ed.), Raven Press, New York (1977), pp. 347-359.

4. R. M. Friedman, E. F. Grollman, **E. H. CHANG**, L. D. Kohn, G. Lee and F. T. Jay. Interferon and glycoprotein hormones. In: Texas Reports on Biology and Medicine (1977), pp. 326-329.

5. R. M. Friedman and **E. H. CHANG**. Interferon action. Possible mechanisms of antiviral activity. In: Interferons and Their Actions (M. Stewart, ed.) CRC Handbook Series (1977), pp. 145-152.

6. **E. H. CHANG,** S. J. Mims, T. J. Triche, and R. M. Friedman. Interferon inhibits mouse leukemia virus release: An electron microscope study. **J. Gen. Viron. 34**: 363-367 (1977).

7. P. K. Y. Wong, P. H. Yuen, R. Macleod, **E. H. CHANG,** M. W. Myers, and R. M. Friedman. The effect of interferon on *de novo* infection of Moloney murine leukemia virus. **Cell 10**: 245-252 (1977).

8. **E. H. CHANG,** M. W. Myers, P. K. Y. Wong, and R. M. Friedman. The inhibitory effect of interferon on a temperature-sensitive mutant of Moloney murine leukemia virus. **Virology 77**: 625-636 (1977).

9. **E. H. CHANG,** and R. M. Friedman. A large glycoprotein of Moloney leukemia vrius derived from interferon-treated cells. **Biochem. Biophys. Res. Commun. 77**: 392-398 (1977).

10. **E. H. CHANG,** F. T. Jay and R. M. Friedman. Physical and morphological alteration in the membrane of AKR cells following interferon treatment and their correlation with the establishment of the antiviral state. **Proc. Natl. Acad. Sci. 75**: 1859-1863 (1978).

11. **E. H. CHANG**, E. F. Grollman, F.T. Jay, G. Lee, L. D. Kohn and R.M. Friedman. Membrane alterations following interferon treatment. In: Human interferon. W. Alton Jones Cell Science Center, Lake Placid, New York (1978), pp. 85-99.

12. A. K. Bandyopadhyay, **E. H. CHANG,** C. C. Levy and R. M. Friedman. Structura abnormalities in murine leukemia viruses produced by interferon-treated cells. **Biochem. Biophys. Res. Commun. 87**: 983-988 (1979).

13. G. L. Hager, **E. H. CHANG,** H. W. Chan, C. F. Garon, M. A. Israel, M. A. Martin, E. M. Scolnick and D. R. Lowy. Molecular cloning of the Harvey sarcoma virus closed circular DNA intermediates: Initial structural and biological characterization. **J. Virol. 31**: 795-809 (1979).

14. H. W. Chan, C. F. Garon, **E. H. CHANG**, D. R. Lowy, G. L. Hager, E. M. Scolnick, R. Repaske and M. A. Martin. Molecular cloning of the Harvey sarcoma virus circular DNA intermediates: II. Further structural analyses. **J. Virol. 33**: 845-855. (1980).

15. A. I. Oliff, G. L. Hager, **E. H. CHANG,** E. M. Scolnick, H. W. Chan and D. R. Lowy. Transfection of molecularly cloned Friend murine leukemia virus DNA yields a highly leukemogenic helper independent type C virus. **J. Virol. 33**: 475-486 (1980).

16. S. L. Berger, M. J. Hitchcock, K. C. Zoon, C. S. Birkenmeier, R. M. Friedman and **E. H. CHANG**. Characterization of interferon messenger RNA synthesis in namalva cells. **J. Biol. Chem. 255:** 2955-2961 (1980).

17. **E. H. CHANG**, J. Maryak, D. M. Wei, T. Y. Shih, R. Shober, H. L. Cheung, R. W. Ellis, G. L. Hager, E. M. Scolnick and D. R. Lowy. Functional organization of the Harvey murine sarcoma virus genome. **J. Virol. 35**: 76-92 (1980).

18. R. W. Ellis, D. DeFeo, J. M. Maryak, H. A. Young, T. Y. Shih, **E. H. CHANG,** D. R. Lowy and E. M. Scolnick. A dual evolutionary origin for the rat genetic sequences of Harvey murine sarcoma virus. **J. Virol. 36**: 408-420 (1980).

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20. E. M. Scolnick, T. Y. Shih, J. Maryak, R. Ellis, **E. H. CHANG** and D. Lowy. Guanine neucleotide binding activity of *src* gene product of rat-derived murine sarcoma viruses. **Ann. N.Y. Acad. Sci. 354**: 398-409 (1980).

21. **E. H. CHANG,** R. W. Ellis, E. M. Scolnick and D. R. Lowy. Transformation by cloned Harvey murine sarcoma virus DNA: Efficiency increased by long terminal repeat DNA. **Science 210**: 1249-1251 (1980).

22. D. DeFeo, M. A. Gonda, H. A. Young, **E. H. CHANG,** D. R. Lowy, E. M. Scolnick and R. W. Ellis. Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus. **Proc. Natl. Acad. Sci. 78**. 3328-3332 (1981).

23. D.R. Lowy, R.W. Ellis, D. DeFeo, **E. H. CHANG**, M.A. Gonda, H.A. Young, N. Tsuchida, T.Y. Shih and E.M. Scolnick. The cellular p21 sarc genes represent a family of divergent normal genes which have the capacity to transform mouse cells. In: RNA Tumor Viruses, New York, Cold Spring Harbor (1981), p. 294.

24. D.R. Lowy, R.W. Ellis, D. DeFeo, **E. H. CHANG**, M.A. Gonda, A. Young, T.Y. Shih and E.M. Scolnick. The family of cellular P21 sarc genes. In: Intl. Union of Microbiol. Soc., Virology Division (1981), p. 462.

25. **E. H. CHANG,** D.R. Lowy, M. Gonda, D. DeFeo, E.M. Scolnick and R.W. Ellis. The p21 gene family: Human and rodent DNA sequences homologous to the transforming genes of Harvey and Kirsten murine sarcoma viruses. In: Advances in Comparative Leukemia Research (1981), pp. 379-380.

26. D. R. Lowy. **E. H. CHANG**, R. W. Ellis, D. Defeo and E. M. Scolnick. Elevated levels of an evolutionarily conserved normal rat protein can induce cellular transformation. **Clin. Res. 29(2):** 428 (1981).

27. S. K. Chattapadhyay, **E. H. CHANG**, M. R. Lander, R. W. Ellis, E. M. Scolnick and D. R. Lowy. Selective amplification of *onc* genes in mammalian species. **Nature 296**: 361-363 (1982).

28. D. R. Lowy, **E. H. CHANG**, R. M. Ellis, M. A. Gonda, T. Shih, D. DeFeo and E. M. Scolnick. Harvey and Kirsten sarcoma viruses and the P-21 gene family. **J. Cell Biochem. Suppl. 6**: 194 (1982).

29. **E. H. CHANG**, M. A. Gonda, R. W. Ellis, E. M. Scolnick and D. R. Lowy. The human genome contains four genes homolgous to the transforming genes of Harvey and Kirsten murine sarcoma viruses. **Proc. Natl. Acad. Sci. 79**: 4848-4852 (182).

30. **E. H. CHANG**, M. A. Furth, E. M. Scolnick and D. R. Lowy. Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus. **Nature 297**: 497-483 (1982).

31. D. R. Lowy, M. A. Gonda, M. E. Furth, R. W. Ellis, E. M. Scolnick, and **E. H. CHANG.** Tumorigenic transformation of mammalian cells induced by elevated levels of a normal human *onc* protein. **Clin. Res. 30(2)**: 421 (1982).

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33. B. D. Crawford, **E. H. CHANG**, J. L. Goodwin, C. E. Hildebrand, P. M. Kraemer, J. L. Longmire and R. D. Palmiter. **J. Cell Biochem. Suppl.** 7: 135 (1983).

34. **E. H. CHANG**, M.A. Gonda, M.E. Furth, J.L. Goodwin, S.E. Yu, R.W. Ellis, E.M. Scolnick and D.R. Lowy. Characterization of four members of the p21 gene family isolated from normal human genomic DNA and demonstration of their oncogenic potential. In: Gene Transfer and Cancer, Raven Press, New York (1983), pp. 189-197.

35. **E. H. CHANG**, M.A. Gonda, E.M. Scolnick and D.R. Lowy. Characterization of 4 divergent human genomic clones homologenes to the transforming p21 genes of Harvey and KiMuSV. In: Gene to Protein--Translation into Biotechnology, American Press (1983), p. 512.

36. D.R. Lowy, M.A. Gonda, M.A. Furth, R.W. Ellis, E.M. Scolnick and **E. H. CHANG**. The human genes homologous to p21 *ras* viral oncogenes. In: Tumor Viruses and Differentiation, Alan R. Liss, Inc., (1983), pp. 435-444.

37. D. Samid, **E. H. CHANG** and R.M. Friedman. Revertants from interferon-treated mouse cells transformed by a human oncogene. In: The Biology of the Interferon System, Elsevier Science Publishers, (1983), pp. 359-360.

38. M. S. McCoy, J. J. Toole, J. M. Cunningham, **E. H. CHANG**, D. R. Lowy and R. A. Weinberg. Characterization of a human colon/lung carcinoma oncogene. **Nature 302**: 79-81 (1983).

39. S. J. O'Brien, W. G. Nash, J. L. Goodwin, D. R. Lowy and **E. H. CHANG**. Dispersion of the *ras* family of transforming genes to four different chromosomes in man. **Nature 302**: 839-842 (1983).

40. M. R. Pincus, J. van Reswoude, J. B. Harford, **E. H. CHANG** and R. D. Klausner. Prediction of the three-dimensional structure of the transforming region of the EJ/T24 human bladder oncogene product and its normal cellular homologue. **Proc. Natl. Acad. Sci. 80**: 5253-5257 (1983).

41. S. J. O'Brien, W. G. Nash, R. Bauer, **E. H. CHANG** and L. J. Seigel. Trends in chromosomal and oncogene evolution in vertebrates. "Uses and Standardization in Vertebrae Culture Cells" (M. K. Paterson, ed.), *IN VITRO* **Monograph No. 5**: Gaithersburg Tissue Culture Association (1984), pp. 204-214.

42. D. Samid, **E. H. CHANG** and R. M. Friedman. Biochemical correlates of reversion in interferon-treated mouse cells transformed by a human oncogene. **Biochem. Biophys. Res. Commun. 119:** 21-28 (1984).

43. D. Samid, **E. H. CHANG** and R. M. Friedman. Inhibition by interferon of transformation induced by a human *ras* oncogene. **Biochem. Biophys. Res. Commun. 126(1):** 509-516 (1985).

44. D. Samid, Z. Schaff, **E. H. CHANG** and R.M. Friedman. Reduction in *ras* expression accompanies phenotypic reversion of interferon-treated, c-Ha-*ras* oncogene transformed mouse cells. In: The Biology of the Interferon System (H. Kirchner and H. Shellekens, eds.), Elsevier, Amsterdam (1985), pp. 189-198.

45. D. Samid, Z. Schaff, **E. H. CHANG** and R. M. Friedman. Interferon-induced modulation of human *ras* oncogene expression. Endocoids. In: Progress in Clinical and Biological Research, Vol. 192 (H. Lal, F. La Bella and J. Lane, eds.), Alan R. Liss, New York (1985), pp. 265-268.

46. D. Samid, **E. H. CHANG** and R.M. Friedman. Specific inhibition by interferon of oncogene-induced transformation. In: Serono Symposia Publications, Vol. 24 (F. Dianzani and G.B. Rossi, eds.), Raven Press, New York, (1985), pp. 425-422.

47. D. Samid, D.M. Flessate, J.J. Greene, **E. H. CHANG** and R.M. Friedman. Mechanisms of Antioncogenic activity of interferon in the 2-5A System: Molecular and clinical aspects of the interferon-regulated pathway. In: Prigin. Clinical and Biological Research, Vol. 202, (B.R.G. Williams and R.H. Silverman, eds.), Alan R. Liss, New York (1985), pp. 203-210.

48. D. Samid, **E. H. CHANG** and R.M. Friedman. Regulation of *ras*-expression by interferon. In: Proc. Asian Congress Pharmacol., (1985), pp. 343-364.

49. **E. H. CHANG**, J.K. Lin, and P. C. Huang, eds. Molecular Biology of Neoplasia. Academia Sinica, 1985

50. **E. H. CHANG**. Viral and cellular oncogenes. In: Molecular Biology of Neoplasia. (E.H. Chang, J.K. Lin and P.C. Huang, eds.) Academia Sinica - Taipei, Taiwan (1985), pp. 191-203.

51. D. Samid, **E. H. CHANG**, and R. M. Friedman. Biological and morphological characteristics of phenotypic revertants appearing in interferon-treated mouse cells transformed by a human oncogene. **J. Exp. Path. 2(3)**: 211-222 (1985).

52. **E. H. CHANG**, P. L. Morgan, E. Lee-Lawlor, K. Pirollo, E. A. White, P. N. Tsichlis and D. H. Patrick. Pathogenicity of retroviruses containing either normal human c-Ha-*ras* 1 or bladder carcinoma EJ/T24 *ras* gene. **J. Exp. Path 2:** 177-190 (1985).

53. R. L. Stallings, R. Black, B. D. Crawford and **E. H. CHANG**. Assignment of *ras* protooncogenes in Chinese hamster: Implications for linkage conservation. **Cytogenet. Cell Genet. 43:** 2-5 (1986).

54. **E. H. CHANG**, R. Black, T. Masnyk and J.B. Harford. Effect of interferon on growth of A431 cells and expression of EGF receptors. In: Advances in Gene Technology: Molecular Biology of the Endocrine System. (D. Puett, *et al*, eds.), Proc. 18th Annual Miami Winter Symposium, 1986, pp. 370-371.

55. **E. H. CHANG**, R. Black, Z.Q. Zou, T. Masnyk, J. Ridge, P. Noguchi and J.B. Harford. Interferon modulates growth of A431 cells and expression of EGF receptors. In: Interferons as Cell Growth Inhibition and Antitumor Factors. (R.M. Friedman, T. Merigan and T. Sreevalsan, eds.), Alan R. Liss, New York (1986), pp. 335-350.

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115. K.F. Pirollo, L. Xu and **E.H. Chang**. Immunoliposomes: A Targeted Delivery Tool for Cancer Treatment in Vector Targeting for Therapeutic Gene Delivery. **(D.T. Curiel and J.T. Douglas, eds.) John Wiley & Sons.** 33-62 (2002)

116. K.F. Pirollo, A. Rait, L. Sleer, and **E.H. Chang**. Antisense Therapeutics: From Theory to Clinical Practice. **Pharmacology and Therapeutics (In Press)**

117. Y. J. Jang, K.F. Pirollo, Z. Hao, Y. Chiang, and **E.H. CHANG**. Restoration of the G_1 Block and Apoptotic Pathway in SCCA of the Head and Neck by Adenoviral Vector Mediated p53 Gene Therapy. **Submitted to Carcinogenesis.**

118. L. Xu, K.F. Pirollo, W.H. Tang, L.M. Xiang, A. Rait, D. Ulick, W.A. Alexander and **E.H. CHANG**. Systemic P53 Gene Therapy Using a Tumor-Targeted Adenoviral Vector Results in Radio/Chemo Sensitization and Long-Term Tumor Regression. **Submitted to Science.**

119. A. Rait, K.F. Pirollo , L. Xu, V. Rait, L. Xiang and **E.H. CHANG**, Antisense HER-2 Oligonucleotides Sensitize Human Breast Cancer to Taxotere *In Vitro* and *In Vivo.* **Submitted to Human Gene Therapy.**

120. K B. Bouker, K.F. Pirollo and **E.H. CHANG**, p53: Culprit or Bystander in the Treatment Failure of Radio/Chemotherapy. **Submitted to JNCI.**

121. M.S. Jhaveri, A.S. Rait, J.B. Trepel, **E.H. CHANG**. Antisense oligonucleotides targeted to the human alpha folate receptor sensitize breast cancer cells to doxorubicin treatment *in vitro*. **Submitted to Molecular Cancer Therapeutics**.

THESIS AND DISSERTATION

1. E. H. CHANG. Adaptation of Grace's continuous lines of insect cells to medium containing heterologous serum. Bachelor's Thesis (U.S. Naval Medical Research Unit No. 2, Fu Jen University, Taipei, Taiwan (1968).

2. E. H. CHANG. Comparative studies of growth patterns of Ganjam Virus in CE, BHK and VERO and *Aedes albopictus* cells. Ph.D. Dissertation, Southern Illinois University, Carbondale, Illinois (1974).

PATENT - APPLICATION FILED

1. c-Raf Transgenic Non-Human Mammals.
2. An Automated Method for the Detection of p53 Mutations.
3. Treatment of Tumors by a Combination of Radiation Therapy and Transduction with Polynucleotide Encoding Wild Type p53.
4. Method of Reversal of Resistance to Radiation Therapy and to Chemotherapy in Cancer Cells Using Sequence-Specific Anti-HER-2 Oligonucleotides.
5. Modified Antisense Nucleotides Complementary to a Section of the Human Ha-*ras* Gene.
6. Targeted Liposome Gene Delivery.
7. Compositions and Methods for Reducing Radiation and Drug Resistance in Cells.
8. Systemic Viral/Ligand Gene Delivery System and Gene Therapy.
9. Ligand-PEG "Post-coated" Cationic Liposomes for Targeted Gene Delivery.
10. Antibody Fragment-Targeted Immunoliposomes for Systemic Gene Delivery.
11. A Simplified and Improved Method for Complexing an Antibody Fragment-Targeted Immunoliposome for Systemic Gene Delivery.

1. Currently Active Support:

1. National Institutes of Health, A Novel Improvement on Radiotherapy for SCCHN
   P.I. 20% Effort on Project
   Project Period: 1 APRIL 1999-31 MARCH 2003
   Total: $286,320

2. Natl. Foundation for Cancer Research, Chemosensitization of Breast Cancer by Systemic Delivery of Anti-HER2 Oligonucleotides
   P.I. 5% Effort on Project
   Project Period: 1 OCTOBER 2000- 30 SEPTEMBER 2003
   Total: $130,435

3. NIH STTR Phase I Application 1R41 CA91660-01A1. Targeting Stealth™ Liposome for Cancer Gene Therapy.
   Jointly with SynerGene Therapeutics, Inc
   10% Effort on Project
   Requested project period: 1 JUNE 2002 – 31 MAY 2003
   Total Requested: $41,143 (Georgetown portion).

4. NIH STTR Phase II Application 2R42 CA80449-2A1 Immunoliposome-Mediated Gene Therapy for Prostate Cancer.
   Jointly with SynerGene Therapeutics, Inc.
   20% effort on project.
   Requested Project Period: 1 SEPTEMBER 2002 – 31 AUGUST 2004
   Total Requested: $169,280 (Georgetown University Portion).

5. NCI, decision Network Program, Transferrin-Liposome (Synerlip) Mediated Systemic Gene Delivery for Human Prostate Cancer.
   P.I.
   Project Period: February 1999-
   The Decision Network has chosen our transferrin-lioposome-p53 complex (Synerlipp53) for further development and testing in Phase I clinical trials by the NCI.

6. NCI, Rapid Access to Intervention Development (RAID) Program, Tumor-Specific Targeting of wtp53 by Anti-Transferrin Receptor Single Chain Antibody: A New Therapeutic Strategy for Prostate Cancer Treatment
   P.I.
   Project Period: 1 APRIL 1999-
   The RAID program does not supply funds to the approved projects. The RAID is designed to accomplish tasks that are rate-limiting in bringing discoveries from the laboratory to the clinic. Thus, in support of this project the RAID program is producing, through the use of NCI's development contracts, GLP/GMP grade reagents including the TfRscFv, the liposome and the wtp53 expression plasmid.

2. Past Support:

1. USUHS, Regulation of the Expression of Human c-ras Genes.
   1 OCTOBER 1982 - 30 SEPTEMBER 1985.
   $60,000 - 3 years. P.I. 10%

2. USUHS, Molecular Cloning of a Tumor Oncogene in a Cancer-Prone Family.
   1 OCTOBER 1985 - 30 SEPTEMBER 1989.
   $154,125 - 4 years. P.I. 10%

3. NIH, Oncogenes (c-ras) in Human Cancer Induction.
   1 MAY 1983 - 30 APRIL 1986.
   $160,000 - 3 years. P.I. 40%

4. Medical Applications of Advanced Laser Technology (MAALT). Probing the Molecular Mechanisms of Carcinogenesis.

1 JANUARY 1986 - 31 DECEMBER 1988.
$150,000 - 3 years. P.I. 10%

5. NIH, Oncogenes in Human Cancer Induction.
1 SEPTEMBER 1986 - 31 DECEMBER 1989.
$258,791 - 3 years. P.I. 40%

6. NIH, a program project. Subproject III. Modulation of Tumor Cell Growth. Program project P.I. Paul O. P. T'so, Johns Hopkins University. Program project. Title: Oligonucleotide Analogs as Antiviral/Anticancer Agents.
1 AUGUST 1986 - 31 DECEMBER 1989.
$145,190 - 3 years. Co-P.I. 15%

7. Medical Applications of Advanced Laser Technology (MAALT). Experimental Therapy of Human Colorectal Tumors.
1 JANUARY 1989 - 31 DECEMBER 1990.
$80,000 - 3 years. P.I. 10%

8. NIH, Modulation of Tumor Growth in vitro and in vivo.
1 JULY 1990 - 30 JUNE 1995.
$649,018 - 5 years. P.I. 15%

9. NIH, Oncogenes in Human Cancer Induction.
1 DECEMBER 1989 - 30 NOVEMBER 1994.
$757,798 - 5 years. P.I. 25%

10. USUHS, Inherited Genetic Defects in Li-Fraumeni Syndrome.
1 OCTOBER 1992 - 30 SEPTEMBER 1995.
$81,000 - 3 years. Co-P.I. 5%

11. Naval Medical Research and Development Command. Demonstration of Cytokines and Growth Factors in Wound Healing.
1 APRIL 1991 - 30 SEPTEMBER 1996
$485,400 – 5.5 years P.I. 5%

12. National Foundation for Cancer Research, HU0001, Modulation of the Radiation-Resistant Phenotypes of Tumor Cells by Sequence-Specific Oligonucleotides.
1 OCTOBER 1988 - 30 SEPTEMBER 1999
$638,750-9 years P.I. 10%

13. NIH, CA45158, The Status of Suppressor Genes in a Cancer-Prone Family.
1 DECEMBER 1994 - 30 NOVEMBER 1999
$1,003,887 – 5 years P.I. 30%

14. Genetic Therapy Inc./NOVARTIS, Sensitization of Tumors to Radiation Therapy by Restoration of the G1 Checkpoint.
1 OCTOBER 1997 - 30 SEPTEMBER 1998
$60,000-1 year P.I. 5%

15. NIH STTR Phase I Application 1 RA1 CA80449-01. Immunoliposome-Mediated Gene Therapy for Prostate Cancer.
(Jointly with SynerGene Therapeutics, Inc.)
1 NOVEMBER 1998 - 31 OCTOBER, 1999
$57,600 (Georgetown University Portion) 1 year, P.I. 10%

16. NIH, 5D50 CA58185-06, SPORE in Breast Cancer (Marc E. Lippman, P.I.).
Development Project, p53 Mediated, Tumor-Targeted Sensitization to Chemotherapy and Radiotherapy.
1 SEPTEMBER 1997 - 31 AUGUST 2001
$50,000- 4 year P.I. 10%

17. DOD Concept Award, Systemic Apoptin Gene Therapy for Chemo/Radiosensitization of Breast Cancer
1 SEPTEMBER 2000-31 AUGUST 2001

$50,000- 1 year P.I. 5%

3. Pending Support

1. NIH RO1 Application. Non-Invasive Methods to Assess p53 Gene Therapy Effects.
Submitted on February 1, 2001
15% effort on project
Request Project Period: 1 DECEMBER 2001 – 30 NOVEMBER 2005
Total Requested: $1,513,200

2. NIH RO1 Application. Surrogate End-Points to Assess p53 Therapy in SCCHN.
Submitted on June 1, 2001
10% effort on project (CO-PI)
Requested Project Period: 1 April, 2002 – 31 March, 2006
Total Requested: $1,496,640

3. NIH RO1 Application. Systematic Sensitization of Pancreatic Cancer to Gemzar
Submitted on February 1, 2002
20% effort on project
Requested Project Period: 1 December 2002 – 30 November 2006
Total Requested: $1,864,810